REMARKS

Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 02-1818. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

A change of address accompanies this response.

Claims 1-3, 5-8, 14, 15, 17-19, 22-33, 43 and 44 are pending. Claims 1, 14, 15, 17, 22-24, 27, 30, 43 and 44 are amended, and claims 9, 10, 20, 21 and 42 are cancelled without prejudice or disclaimer. Independent claims 1 and 27 are amended to address the rejection under 35 U.S.C. §112, second paragraph, to render it clear that amino acids are replaced one-by-one along the full length of the protein to be evolved. In addition, the amendment to recite that each amino acid along the full-length of the protein is replaced should moot the rejections under 35 U.S.C. §103(a).

Claim 1 also is amended to include the limitations of claims 9 and 10. These amendments are not to be construed as a concession that the rejections are proper, but to advance claims to issue. The claims are amended for consistency and to simplify the claim structure by rewriting some independent claims as dependent clams. The application should be in condition for allowance.

Applicant reserves the right to file divisional applications to any cancelled or previously unclaimed subject matter. Also, Applicant points out that claim 1 previously was amended by incorporation of previously pending (cancelled herein) claims 20 and 21. The recitation that the amino acids are replaced along the full-length of the protein (or along the length of a domain) has been pending in the application throughout the prosecution. Thus the limitations "of producing proteins that differ by one amino acid along the full length of a target protein or a pre-selected domain of the target protein" noted by the Examiner to necessitate the new ground of rejection previously were pending. Thus, the new grounds of rejection under 35 USC §103(a) are not necessitated by amendment. Also, amendment of the claims to recite "whereby the identity of each set of nucleic acid molecules in host cells at each locus in the array is known" to render it clear that as intended in the claim and by virtue of the method an array is produced in which the identity of the members is known a priori. This inherent limitation and element has been argued throughout the prosecution; and, thus was not new the to previous response.

In an effort to advance this application to allowance, however, Applicant has not filed a Petition to remove finality, but rather is filing an RCE within two months of receipt of the Office Action with the goal of advancing claims to allowance.

THE REJECTIONS OF CLAIMS 1-3, 5-10, 14, 15, 17-33, and 42-44 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1, 22, 23, 25, 27 and 30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of "the encoded amino acid residues are each replaced along the full-length of the encoded protein or along the full length of a pre-selected domain of the encoded protein so that all position along the full-length or a domain of the protein are individually modified for screening" because it allegedly contradicts the requirement that the encoded proteins differ from other encoded proteins by only one amino acid. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

It respectfully is submitted that as previously pending the claims recited that

> the encoded amino acid residues are each replaced along the full-length of the encoded protein or along the full-length of a pre-selected domain of the encoded protein **so that all positions along the full-length** or a domain of the protein are **individually modified f**or screening. [emphasis added]

Thus the claims recite that each encoded residue is replaced along the full-length **so that all positions . . . are individually modified.** Nevertheless, in order to place the claims into condition for allowance the claims are amended to recite

> **a sufficient number of sets of nucleic acid molecules are produced so that each encoded amino acid residue in the encoded protein is replaced** ~~the encoded amino acid residues are each replaced~~ along the full-length of the encoded protein ~~or along the full-length of a pre-selected domain of the encoded protein~~ so that all positions **along the full-length** ~~or a domain~~ of the protein **are individually modified** for screening**, and each nucleic acid molecule encodes a protein that differs by one amino acid from the target protein** . . .

Thus, the claim recites that nucleic acid molecules are individually modified and each nucleic acid molecule encodes a protein that differs by one amino acid from the target protein, rendering it clear, if there was any ambiguity, that only one residue is changed on each molecule to be screened and that all loci are assessed. The Examiner also is reminded that claims are read in light of the specification, which renders it clear that only one amino acid is changed at a time. If any concerns remain regarding this language, the Examiner is encouraged to call the undersigned to discuss alternative language.

The other amendments are designed to simplify the claim structure by rewriting independent claims as dependent claims, correcting some obvious inconsistencies in claim language, and cancelling claims whose limitations are reflected in the independent claims. Claim 1 also is amended to include the limitations of claims 9 and 10, which are cancelled herein.

THE CLAIMED SUBJECT MATTER

Before addressing the rejections under 35 U.S.C. §103, the subject matter of the claims is discussed. It is believed that the claims adequately reflect each step noted below. The method is a for modifying a target polypeptide to have a change in a **predetermined** property or activity. The method produces Lead proteins that have a predetermined evolved property or activity The method involves first replacing every amino acid along the full-length of the protein with a pre-selected amino acid and testing each resulting protein for the property or an activity to identify any that have a change in the activity or property. This changing and testing and is effected along the full length of the protein and is done individually, albeit in a high throughput format, each protein is separately produced and separately screened so that every residue along the full-length of the protein is tested to identify hits. As a result there is no bias created by screening mixtures of molecules or creating mixtures so that all hits are identified without any bias that occurs when mixtures of mutations are added or multiple mutations are included or mixtures are screened..

In the second part fo the method , each hit locus is then individually replaced with every other amino acid and again individually screened to identify leads; leads differ in the predetermined activity or property from the hit, typically exhibiting a desired activity or property. Again bias in production, expression and/or screening, is avoided because all modifications at hit positions will be individually expressed and screened. In addition, because each modified protein is individually modified at a single separate locus, the identity of the protein at each locus is known prior to screening, so that upon identification of a hit, no sequencing or other identification is needed. The method is amenable to high throughput screening.

As shown in the application and discussed previously, the application exemplifies practice of the method with AAV Rep protein-encoding nucleic acid to identify mutations that result in production of higher titer AAV. While AAV viruses with mutated Rep proteins previous have been produced, **none** resulted in higher titer. Application of the instantly claimed method, in which every position along the length of the nucleic acid molecule was replaced and tested to identify hits, and then each hit was replaced with every other amino acid, however, resulted in identification of Rep protein mutations that result in higher titer. A least 6 mutations that result in higher titer of AAV virus were identified, thereby demonstrating the power of the method. Because the method essentially tests every residue to identify those that alter a particular activity or property and the screens every replacing amino acid at those positions and does so individually, it ferrets out valuable

mutations that are missed by prior art methods that somehow limit or bias the screening, such as by expressing or screening mixtures or failing to test all loci in the protein.

During the extensive prosecution of this application, no art, singly or in combination, that teaches the method as claimed as been identified. All rejections of record have been obviated. As discussed below, the currently cited art, new applied, to the extent pertinent, only are pertinent to modification of domains of a protein. In an effort to advance subject matter to allowance, while none of the art, singly or in combination teaches or suggests, the embodiments, directed to modifying a domain of a protein, the claims as amended, do require modification of every residue along the full-length of the target protein. None of the cited art contemplates directed evolution, and none contemplates a directed evolution method in which the full-length of a target protein is modified as claimed. Therefore, as discussed below, the outstanding rejections should be moot.

THE REJECTIONS OF CLAIMS 7, 24-29, 32, 33 UNDER 35 U.S.C. §103(a)

Several grounds of rejection are set forth under 35 U.S.C. §103(a) The particular grounds of rejection are discussed in turn below. As discussed below, none of Ladner *et al.*, Wells *et al.*, and Pederson *et al.*,, singly or in combination, nor any reference of record, nor any combination of references, teaches or suggests steps (a) -(d) nor steps (a)- (g) of claim 1 and dependents nor steps (a)-(f) of claim 27 and dependents as outlined above and as recited in each of the independent claims. None of the references teaches or suggests changing one amino acid at a time along the full length of the protein such that each protein with a single change is individually produced, expressed and screened so that **all** hits along the full-length or domain are identified. None suggests separately introducing each nucleic acid molecules cells of one locus of an array to produce an addressable array in which the identity of the encoded protein is known *a priori*. None suggests then, after identifying hits by screening every amino acid position along the full length of the protein, individually modifying, expressing and screening each hit with every amino acid (remaining) to identify the particular changes that lead to the desired change in activity or property (i.e. the evolved property).

In addition, as exemplified in the application, the methods of the instant application are very powerful, permitting preparation of modified polypeptides that have a predetermined property or activity. In the working example in the application, the overlapping Rep protein-encoding gene(s) of AAV are changed one codon at a time along the full-length of the open reading frames encoding the Rep proteins to identify loci (hits) whose modification alters AAV titer. Each hit is then changed to every other amino acid to identify modified

proteins that result in higher titer. The methods identifies at least 6 such proteins in each serotype. Heretofore, no Rep mutants had ever been identified that result in increased titer. Hence the method permits evolution of protein (*i.e.*, Rep proteins) to exhibit a predetermined property (*i.e.*, higher titer).

As discussed below, the Ladner *et al.*, fails to teach **any** steps or elements of the methods as claimed. Ladner *et al.* does not teach or suggest individually modifying only one amino acid of encoded proteins and individually screening each. The method of Ladner *et al.* is designed to produce mixtures of nucleic acid molecules and proteins. Ladner *et al.* specifically teaches modifying 5-10 amino acids at a time. Ladner *et al.* does not teach or suggest modifying amino acids along the full length of a target protein. The teachings of Wells *et al.* and/or Pederson *et al.* does not cure these deficiencies. Wells *et al.* does not teach or suggest modifying a protein along its full-length, as Wells *et al.* is directed to a method for analysis of the structure and function of polypeptides by identifying active domains which influence the activity of the polypeptide with a target substance. The instantly claimed method, in which the protein is modified along its full length does not involve identifying active domains; Wells *et al.* does not teach any of steps (a)-(d) of any pending claims, nor steps (a)-(g) (claim 1) or (a)-(f) (claim 27) of the pending claims and thus, cannot and does not cure the deficiencies in the teachings of Ladner *et al.* Pederson is merely directed methods for creating and screening spatial arrays that contain a single cell at each locus. Pederson is of no relevance to the instantly claimed methods; the instant claims do not require any method for spatially arraying a single clone. In the instantly claimed methods, since each nucleic acid molecule is individually produced, and separately arrayed, there is no need to clonally select. Further, the method of Pederson does not result in an addressable array in which the identity of each clone at each locus is known *a priori*. Picking a clone from among a mixture and arraying each as in Pederson, does not result in an array in which the identity of each protein at each locus is known *a priori*. Thus, as discussed below, the combination of teachings of the references does not teach or suggest or result in the instantly claimed references.

RELEVANT LAW

To establish prima facie obviousness under 35 U.S.C. §103, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This principle of U.S. law regarding obviousness was not altered by the recent

Supreme Court holding in KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 82 USPQ2d 1385 (2007). In KSR, the Supreme Court stated that "Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior art are such the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art. Graham v. John Deere Co., 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966). See also KSR, 127 S.Ct. at 1734, 82 USPQ2d at 1391 ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.") The Court in Graham noted that evidence of secondary considerations, such as commercial success, long felt but unsolved needs, failure of others, etc., "might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented." 383 U.S. at 18, 148 USPQ at 467. Furthermore, the Court in KSR took the opportunity to reiterate a second long-standing principle of U.S. law: that a holding of obviousness requires the fact finder (here, the Examiner), to make explicit the analysis supporting a rejection under 35 U.S.C. 103, stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. Id. at 1740-41, 82 USPQ2d at 1396 (citing In re Kahn, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006)).

While the KSR Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. KSR, 127 S. Ct. at 1731. The court stated in dicta that, where there is a "market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of

innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try **might** show that it was obvious under § 103."

In a post-KSR decision, PharmaStem Therapeutics. Inc. v. ViaCell. Inc., 491 F.3d 1342 (Fed. Cir. 2007), the Federal Circuit stated that:

> an invention would not be invalid for obviousness if the inventor would have been motivated to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. Likewise, an invention would not be deemed obvious if all that was suggested was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

Furthermore, KSR has not overruled existing case. See In re Papesch, (315 F.2d 381, 137 USPQ 43 (CCPA 1963)), In re Dillon, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991), and In re Deuel (51 F.3d 1552, 1558-59, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995)). "In cases involving new compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound." *Takeda v. Alphapharm*, 492 F.3d 1350 (Fed. Cir. 2007).

The mere fact that prior art may be modified to produce what is claimed does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, *In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

As always, unexpected properties must always be considered in the determination of obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesch*, 315 F.2d 381, 391, 137 USPQ 43, 51 (CCPA 1963)

The disclosure of the applicant cannot be used to hunt through the prior art for the claimed elements and then combine them as claimed. In re Laskowski, 871 F.2d 115, 117, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey

or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

In the instant case, the combination of teachings of the references does not teach or suggest all elements of the methods as claimed, the proposed combination would necessarily change the operation of the prior art method, and none of the cited references nor any combination thereof teaches or suggests changes in the cited art that would result in the instantly claimed methods. To combine the references to produce to claimed methods would require use of the instant application as a guide, involving the impermissible use of hindsight.

Rejection of claims

Claims 1-3, 5-10, 14, 15, 17-23, 27, 43 and 44 are rejected under 35 U.S.C. §103(a) as being unpatentable over Ladner et al. (US 5,223,409) in view of Wells et al. (US 6,013,478) and in view of Pedersen et al. (WO01/32844). The Examiner urges that:

> Ladner et al. teaches a method of producing a plurality of separate sets of nucleic acid molecules that encode modified forms of a target protein wherein the nucleic acid molecules in each set are produced by changing codons; and each set of molecules encode a proteins that differ from proteins encoded by other sets by the changed codons. Ladner et al. further teaches that each molecule may be individually introduced into a host cell where the identity of the nucleic acid molecule is known.

The Examiner then states that Ladner et al. does not teach modifying one amino acid at a time nor using an addressable array, but that Wells et al. and Pederson et al. cure these deficiencies. The Examiner states that Wells et al. teaches a method "where a residue substituted DNA sequence encodes a residue substituted polypeptide where each polypeptide contains a single substitution at a different amino acid within the active domain (i.e. where each protein encoded by a nucleic acid molecule set differs from the other proteins by only one amino acid and where the amino acid residues are replaced along the full-length of the pre-selected domain)." The Examiner states that Pederson teaches an addressable array. The Examiner concludes:

> It would have been obvious to one of ordinary skill in the art at the time of the invention to combine the methods of Ladner et al., Wells et al., and Pedersen et al. to gain the benefit of identifying individual active amino acid residues as well as a high throughput screening method. Ladner et al. teaches a generic method of utilizing directed evolution of a target protein. However, Wells et al. teaches that in order to identify the individual amino acid residues involved in a binding domain, a plurality of residue substituted polypeptides are created,

> each containing a single substitution of an amino acid residue within the active domain (column 17, line 60-column 16, line 11). Thus one of ordinary skill in the art seeking to use Ladner et al.'s method to identify active amino acid residues, would initially create polypeptides that differ by one amino acid at the binding domain. Furthermore, in order to screen all of these polypeptides, one of ordinary skill in the art would be motivated to use a high-throughput screening method as taught by Pedersen et al. which offers the advantage of a practical and reliable method for the identification of novel substances with new properties from a large number of molecules (page 37, lines 19-24). Thus, one of ordinary skill in the art would have been motivated to combine the methods of Ladner et al., Well et al. and Pedersen et al. for the reasons above.

This rejection respectfully is traversed.

The rejected claims

The claims are discussed above. As amended claims 1 and 27 are independent claims each reciting all steps to produce leads. Claim 1 recites:

> A process for the identification of a protein that differs in a predetermined property or activity from a target protein, comprising:
>
> (a) producing a plurality of separate sets of nucleic acid molecules that encode modified forms of a target protein, wherein:
>
> the nucleic acid molecules in each set are produced by changing one codon in the target protein to a pre-selected codon, whereby the nucleic acid molecules in each set encode proteins that differ from the encoded proteins in another set by one amino acid;
>
> a sufficient number of sets of nucleic acid molecules are produced so that each encoded amino acid residue in the encoded protein is replaced with a pre-selected amino acid along the full-length of the encoded protein so that all positions along the full-length of the protein are individually modified for screening, and each nucleic acid molecule encodes a protein that differs by one amino acid from the target protein; and
>
> all nucleic acid molecules in a set encode the same modified protein;
>
> (b) individually introducing each set of nucleic acid molecules into host cells to produce an addressable array of host cells, whereby the identity of each set of nucleic acid molecules in host cells of each locus in the array is known, wherein the cells of each locus of the addressable array contain the same modified nucleic acid molecules;
>
> (c) expressing the encoded proteins, whereby a plurality of separate sets of proteins encoded by the nucleic acid molecules are produced, wherein:
>
> all of the encoded proteins in each set have the same modification; and
>
> the proteins in each set differ from the proteins in another set by one amino acid and from the target protein by one amino acid;
>
> (d) individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target , wherein:
>
> each identified protein is designated a hit;
>
> each hit contains a mutation designated a hit position; and

the predetermined property or activity is selected from among a chemical, a physical and a biological property or an activity of the target protein;

(e) modifying the nucleic acid molecules that encode the hits to produce sets of nucleic acid molecules that encode modified hits, wherein:

the modified hits are produced by systematically and individually replacing each codon that is a hit position with a codon encoding another amino acid to produce nucleic acid molecules each differing by at least one codon and encoding modified hits; each set of nucleic acid molecules is individually designed and synthesized., whereby:

a sufficient number of sets are produced to produce encoded proteins in which every hit is separately replaced with all other amino acids, and the encoded protein in each set differs from the encoded protein each other set and the target protein by one amino acid ;

the identity of each set of nucleic acid molecules in host cells of each locus in the array is known and wherein the cells of each locus of the addressable array contain the same modified nucleic acid molecules;

(f) separately introducing each set of nucleic molecules that encodes the modified hits into cells to produce an addressable array of cells; and

(g) individually screening each set of cells that contains the nucleic acid molecules that encode the modified hits to identify one or more nucleic acid molecules that encode(s) a protein or the coded protein that has/have a predetermined property or activity that differs from the target protein and has properties that differ from the original hits, wherein each such protein is designated a lead.

Claim 27 recites:

A process for the identification of a protein that differs in a predetermined property from a target protein, comprising:

(a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein:

each encoded modified protein in a set differs from the encoded proteins in each other set and from the target protein by one amino acid;

a sufficient number of sets of nucleic acid molecules are produced so that each encoded amino acid residue in the encoded protein is replaced with a pre-selected amino acid along the full-length of the encoded protein so that all positions along the full-length of the protein are individually modified for screening, and each nucleic acid molecule encodes a protein that differs by one amino acid from the target protein; and

the members of each set encode the same modified protein;

(b) individually introducing each set of nucleic acid molecules into host cells and expressing the encoded protein, wherein:

the host cells are organized in an addressable array, whereby the identity of each nucleic acid molecule at each locus in the array is known;

each set of nucleic acid molecules is introduced into host cells at a different locus of the array, whereby the identity of each set of nucleic acid molecules in host cells at each locus of the array is known, wherein:

the cells of each locus of the addressable array contain the same modified nucleic acid molecules;

all encoded proteins in each set contain the same modification; and

the proteins in each set differ from the proteins in another set by one amino acid and from the target protein by one amino acid;

(c) individually screening the sets of encoded proteins to identify one or more proteins designated hits$$$ that have a predetermined property that differs from the target protein are identified, wherein:

each identified protein is designated a hit;

each hit contains a mutation designated a hit position; and

the predetermined property is selected from among a chemical, a physical and a biological property of the target protein;

the nucleic acid molecules comprise viral vectors; and the cells are eukaryotic cells that are transduced with the vectors;

(d) modifying the nucleic acid molecules that encode the hits to produce a set of nucleic acid molecules that encode modified hits, wherein each nucleic acid is in a viral vector,;

(e) introducing each set of nucleic acids that encode the modified hits into cells; and

(f) individually screening the sets of cells that contain the nucleic acid molecules that encode the modified hits to identify one or more cells that encodes a protein that has a predetermined property or activity that differs from the target protein and has properties that differ from the original hits, wherein each such protein is designated a lead.

As discussed above, all of the claims are directed to methods of directed evolution in which a target protein is modified one amino acid at a time along its full length, and each modified protein is separately expressed and screened separately from the other modified proteins. One amino acid change is introduced per protein, not one or more changes, but one change. Mixtures of different proteins are not produced, nor are mixtures screened. In addition, because one change is made at a time and the nucleic acid molecules encoding each protein are introduced host cells at different loci in an array the identity of the nucleic acid at each locus in the array, and, thus the protein, is known. The identified hits are then further modified by replacing each hit with other amino acids (claims 1 and 27), including all amino acids (claim 1) and each such further modified hit is individually produced and screened.

As described in the application the method does not rely on any methods in which there is differential modification or expression of a particular modified protein. For example, at page 24, lines 6-24, the specification states:

> The whole process of the identification of the active site(s) on the full length protein sequence requires the following sub-steps:
>
> a. Generation of a mutant library (on the gene to be evolved) in which each individual mutant contains a single mutation located at a different amino acid position and that includes a systematic replacement of the native amino acid by Ala or any other amino acid (always the same throughout the entire protein sequence);

b. phenotypic characterization of the individual mutants, one-by-one and assessment of mutant protein activity;

c. identification of those mutants that display an alteration, typically a decrease, in the selected protein activity, thus, indicating that amino acids directly involved in the active site(s) have been hit. The aa positions whose aa-scan mutations display an alteration, typically a loss or decrease, in activity are named HITS.

The identification of the active site(s) (HITS) is thus, by this method, made in a completely unbiased manner. There are no assumptions about the specific structure of the protein in question nor any knowledge or assumptions about the active site(s). The results of the amino acid scan identify such sites.

Similarly, after identification of hits, each and every hit is replaced with every amino acid and each such-modified protein is separately expressed and screened. As a result no bias is introduced by virtue of modification, expression or screening.

Hence, there is no bias introduced into the process. Amino acids are rationally replaced and each variant is tested separately. There is no effect of differential expression in culture in which conditions could favor one variant over another nor effects of differential mutation, in which one locus might be more amenable to modification. Further, as exemplified, the method is quite powerful, permitting evolution, for example, of AAV Rep proteins to produce modified AAV that has increased titer. Further the method identified all modifications in the protein that result in increased titer. While modifications in the viral Rep genes was known, there had been no mutations identified that result increased titer. The instantly claimed unbiased systematic rational method permitted mutations that result in increased titer to be identified.

Teachings of each cited reference and differences from the claimed methods

Ladner *et al.*

Ladner *et al.* describes a methods that involve controlled random mutagenesis to produce **a mixture** of nucleic acid molecules that encode different but related proteins. The **mixture** of nucleic acid molecules is introduced into a genetic package, such as a virus, such that the encoded proteins are expressed (displayed) on the surface of the virus to produce a **mixture** of viruses that display the encoded proteins. In the method, the genetic packages first display an initial potential binding domain (IPBD) to confirm that it binds to the target.

Once the IPBD is identified, it is subjected to a multiple mutagenesis protocol called **variegated mutagenesis**, which by its nature necessarily results in mixtures that include proteins with one or more modifications, to produce a **population** of genetic packages that display the potential binding domains (PBDs), which are the mutated IPBDs. Thus, Ladner

et al. describes a method in which mixtures of nucleic acids are produced, the nucleic acid molecules encode modified proteins that can differ in more than one amino acid (typically 5-10 residues are varied at a time), and the mixtures are screened.

Ladner *et al.* teaches of selection of residues to vary, not by testing every residue, but only those contemplated to be involved in binding of the target protein based on the 3D structure of the protein, sequences of homologous proteins, or on computer modeling. Further, a hallmark of the method involves **simultaneously varying** a plurality of residues. Typically, 5-10 residues are varied at any one time and mixtures of modified polypeptides are produced. This is exemplified in the specification and in the Examples where, for example, 4-6 residues (see e.g., column 32, line, 24), 5-10 residues (see e.g., column 32, line 34 and line 46), 6 residues (Example IV, column 134, lines 29-63), or 5 residues (column 144, lines 10-20) are described as being varied at one time. For example, column 31, lines 39-53 describes the selection and variegation of residues as follows:

> There are many ways to generate diversity in a protein. (see RICH86, CARU85 and OLIP86.) At one extreme, we vary a few residues of the protein as much as possible (inter alia see CARU85, CARU87, RICH86, and WHAR86). We will call this approach "Focused Mutagenesis". A typical "Focused Mutagenesis" strategy is to pick a set of five to seven residues to vary each through 13-20 possibilities. An alternative plan of mutagenesis ("Diffuse Mutagenesis") is to vary many more residues through a more limited set of choices (see VERS86a and PAKU86). The variegation pattern adopted may fall between these two extremes, e.g., two residues varied through all twenty amino acids, two more through only two possibilities, and a fifth into ten of the twenty amino acids.

The variegated method **necessarily** produces libraries (mixtures) of phage displaying the variegated polypeptides on their surfaces and produces mixtures in which each protein differs from the target by 5 to 10 amino acid residues. . Using affinity selection, these libraries are then screened **as mixtures** for binding to a target to enrich for phage that express proteins that exhibit improved binding to a target material. Such bound phage are recovered and enriched phage are amplified by culture in suitable medium. Clonal isolates are obtained. The identity of each nucleic acid molecule in the clonal isolate **is not known**. The isolates are characterized by genetic methods, such as sequencing to identify, the identity of the mutated residues that confer the improved binding of the protein to the target.

Thus, Ladner *et al.* teaches a method that is **completely** different from the instantly claimed methods. In the method of Ladner *et al.* a target protein is modified by producing

mixtures in which 5-10 amino acid residues in each protein in the mixture are changed simultaneously and the **mixtures are screened testing clonal isolates.** Testing a clone isolate is not the same as individually modifying a protein so that the identity of the protein is known. A clonal isolate may include only a single variant, but the identity of the variant is **not known.**

Ladner *et al.*, does **not** teach or suggest that the mutations are produced one-by-one, such that each variant nucleic acid encodes a mutant protein that differs by one replacement amino acid from the original protein. In Ladner, a plurality of mutations are introduced into one molecule. Ladner *et al.* does not teach or suggest modifying the protein along its full length with a pre-selected amino acid; nor does Ladner *et al.* teach or suggest part two of the instantly claimed method in which each residue is replaced by all other amino acids; again replacement is one-by-one and each resulting protein is individually screened.

Further, Ladner does **not** teach or suggest individually introducing each set of nucleic acid molecules into host cells and expressing the encoded proteins in addressable arrays, whereby the identify of the encoded protein at each locus is known and where each protein in a set contains the same amino acid replacement. In Ladner *et al.*, the variegated DNA molecules are provided as a mixture of phage, which are then screened to enrich for phage that express the proteins that bind to the target. Hence, **mixtures** (libraries) of modified proteins are screened; modified proteins are not screened one-by-one.

In addition, because they are screened as a mixture, the identity of the bound proteins are not known; further sequencing of amplified clonal isolates is needed to identify the mutated residues. In the instant methods, the identity of each modified protein is known by virtue of the practice of the method and the individual expression of each protein in an addressable array.

Hence, among the deficiencies in the teachings of Ladner *et al.* is that Ladner *et al.*, fails to teach or suggest introducing mutations into the protein one-by-one and individually screening the mutations, fails to suggest introducing the mutations along the full-length of the protein (or even along a domain) and certainly not one residue at a time, fails to teach or suggest identifying the modified protein by virtue of its locus in an addressable array. Thus, Ladner *et al.* is of little relevance to the instant claims. Neither Wells nor Pederson, singly or in combination, cure the deficiencies in the teachings of Ladner *et al.*.

Wells *et al.*

Wells *et al.* teaches a method for identifying active domains in a protein, and amino acid mutation of residues in the active domain to generate variant proteins with altered activity. Wells *et al.* is concerned with domains, not the full-length polypeptide. In Wells *et al.*, the active domains are identified by substitution of regions of the protein with one or more analogs of the polypeptide of interest, which analogs exhibit a different activity from the polypeptide. These segment-substituted polypeptides are produced and contacted with a target for the parent polypeptide to determine if the substituted region alters the interaction with the target. The active domain of the polypeptide is identified to be those segment substituted regions that confer a change in activity of the segment substituted polypeptide, such as a change in the Kd, relative to a parent polypeptide. In the method of Wells, once the active domain is identified, a scanning amino acid is substituted for an amino acid in an active domain of the parent polypeptide. A plurality of residue-substituted polypeptides are prepared, which each contain a single substitution in a different amino acid residue of the active domain with the same scanning amino acid. A plurality of polypeptides containing substituted residues are prepared and screened.

The residue-substituted polypeptides are then tested for activity with a particular target compared to the parent polypeptide to determine the amino acid residues in the active domain that are involved in the interaction with the target. Further, once the active amino acids in the active domain are identified they can be further modified to alter the interaction of the parent protein with one or more target.

The methods of Wells *et al.* is exemplified by substitution and modification of human growth hormone to alter its interaction with its somatogenic receptor in order to assess the structure/function relationship. In determining the active domain amino acid residues, three active domains were identified based on segment-substituted polypeptides. Residues in the three active domains were then replaced sequentially with alanine, yielding a total of 63 mutants. Residues that alter the interaction with the somatogenic receptor were further modified in the human growth hormone polypeptide.

Wells does not teach or suggest a high throughput method of directed evolution. Wells studies proteins individually in order to indentify active domains of a protein. Wells does not teach or suggest replacing amino acids along the full-length of a protein and testing each one-by-one to identify hits. Wells does not teach the further steps of then replacing the amino acids **at each of the hit loci, one-by-one, with every other amino acid** to identify to

amino acids that confer a desired change in activity or property the protein. Wells *et al.*, identifies and studies active domains. Wells *et al.* does not teach or suggest a method in which the full length of protein is assessed by changing each residue, one-by-one, and then, replacing the amino acids at each and every hit with all amino acids.

Thus, Wells *et al.*, does not cure the deficiencies in the teachings of Ladner. Further, Wells is not properly combinable with Ladner *et al.*, (nor Pederson *et al.*) since the methods in each bear no relationship to the other. Even assuming motivation to combine these references post-KSR, the methods in each cannot be combined in any meaningful way. Ladner *et al.* discloses a method of variegated mutagenesis in which a plurality of residues are modified; Wells is a method or identifying active domains, and prepares proteins with replaced domains to assess the effects, identifies domains of interest and modifies amino acids in the domains. This method has nothing in common with the method of Ladner *et al.*

Pederson *et al.*

Pederson *et al.*, does not cure these deficiencies. Pederson *et al.* teaches a screening method that uses a spatial array to separate molecules in each position of the array for screening. The identify of the molecule in each position in a spatial array is not known **but must be** determined by various screening assays. Pederson *et al.*, teaches that by using this method, variation in expression level still exist, but that uniform growth of identical clones in different wells is achieved. In this instance then, the spatial array is not being used to individually introduce and express mutant proteins, but instead is being used to individually grow cells and express the mutant proteins. The identity of each clone in the array is not known *a priori* and each clone must be further characterized, such as by sequencing to identify the encoded protein.

Hence, Pederson does not teach or suggest using an addressable array for the individual introduction and expression of mutant proteins. The teachings of Pederson point to the fact that the protein molecules are not individually expressed in host cells in the array. Rather, Pederson teaches, such as in Example 5 (see e.g., at page 54, line 14), that spores already expressing **a mixture** of mutants from a mutant library are introduced into A spatial array for screening. For example, distinct mutant **pools** are added to each array so that one spore in average is inoculated per well meaning that some wells will contain no spores and some wells will contain more than one spore. Thus single clones are arrayed, but the are picked from mixtures. As a result, one does not *a priori* know the identity of a spore or

spores in a particular locus in the array (hence it is not "addressable" as required in the instant claims). Example 9 describes another strategy ,which also does not teach or suggest that mutants are individually introduced into host cells, as required by the instant claims. Example 9 (see e.g., page 60, beginning at line 26), describes the generation of a mutant library of lipase, which library is transformed into fungal protoplasts as a mixture using alginate balls. Hence, mixtures of mutants are produced.

Also, in the methods of Pederson, the mutants expressed in each array are not ones that have been generated one-by-one such that each variant nucleic acid encodes a mutant protein that differs by one replacement amino acid from the original nucleic acid molecule. For example, Example 5 (see e.g., page 54, beginning at line 29) teaches the generation of mutant Shearzyme®. As taught in Pederson, random mutants of Shearzyme® are generated by UV-irradiation. Thus, the identity and number of mutations in each mutant protein is not known without further sequencing. Another example, Example 9 teaches an error-prone PCR-based mutation of lipase, which also results in non-targeted, randomized mutation of lipase to generate a **library** of mutants. Thus, Pederson *et al.* does not teach or suggest any elements of the instantly claimed method and does not cure the deficiencies in the teachings of Ladner *et al.* and /or Wells *et al.*

Analysis

The combination of teachings of the cited references fails to result in the method of any pending claim. Hence, neither Pederson *et al.*, nor Ladner *et al.*, nor Wells *et al.*, singly nor in any combination, teaches numerous elements of the instantly claimed methods. As discussed above Ladner *et al.* fails to teach any steps in the instantly claimed method, including: (1) introducing mutations into a protein one-by-one along the full-length of the protein and individually expressing each protein; (3) producing a population of sets of nucleic acid molecules where each set encodes a protein that differs by one amino acid from a target protein; individually screening the mutant proteins; (5) identifying the modified protein by virtue of its locus in an addressable array; and (6) individually replacing the hit amino acids with all other amino acids and individually expressing and screening the proteins with modified hits. Wells *et al.* does not cure these deficiencies. As discussed above, Wells *et al.* is concerned with identifying active domains, and its method requires substitution of a putative domain with a domain from an analogous protein and assessing its effect. Once a domain is identified, amino acids in the domain are modified to assess their effects. Wells *et*

al., however, provides not teachings or suggestions any of (1)-(6). Pederson *et al.* similarly fails to teach any of these elements.

Therefore, the combination of teachings of these references does not and cannot result in the instantly claimed method. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness.

In addition, each reference is directed to a method that is separate and distinct from the other and not amenable to modification. The method of Ladner *et al.* requires the use of variegated nucleic acids and the production of mixtures. The variegated nucleic acids contain a plurality of modifications per molecule. Further the variegated nucleic acids necessarily are produced as a mixture. Wells *et al.* is studying protein domains. There is no way that its method could be combined with Ladner *et al.* to produce the instantly claimed method. Pederson *et al.* is directed to a method involving clonal arrays, in which clones are picked from mixtures and arrayed separately. In the methods described by Pederson, mixtures of clones are produced. There is no teaching or suggestion of any steps in the instantly claimed methods. Therefore, Pederson *et al.* doe s not cure these deficiencies.

Remaining claims

The remaining claims are rejected over combinations of Ladner *et al.,* Wells *et al.* and Pederson *et al.* in view of further references alleged to teach elements of dependent claims, not the independent claims, these rejections fail since dependent claims necessarily include all limitations of the independent base claims, and Ladner *et al.*, Wells *et al.* and Pederson *et al.*, fail to teach or suggest the methods of the independent claims.

Claims 24, 28 and 29

Claims 24, 28 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. (US 5,223,409) in view of Wells et al. (US 6,013,478) in view of Pedersen et al. (W001/32844) as applied to claims 1-3, 5-10, 14, 15, 17-23, 27, 43 and 44 above, and further in view of Berlioz et al. (US 5,925, 565) because Berlioz *et al.* teaches "assessing the titer of the viral vectors after transfection for each set of eukaryotic cells (column 14, lines 39-65) and where the viral vector encodes for a protein involved in viral replication (column 5, lines 35-65)." The Examiner concludes that it:

> would have been obvious to one of ordinary skill in the art at the time of the invention to modify the methods taught by Ladner et al., Wells et al., and Pedersen et al. with Berlioz et al. in order to study the effects of the protein in an eukaryotic setting. Berlioz et al. teaches a method that allows

> eukaryotic cells, such as a human cell, to express a desired protein (column 6, lines 5-22) for the purpose of producing a therapeutic treatment (column 7, lines 15-25). Ladner et al., Wells et al., and Pedersen et al. methods teach screening for different proteins that exhibit a desired biological, chemical, or physical property. Thus one of ordinary skill in the art seeking to create a new therapeutic treatment, would be motivated to use Giver et al.[*sic*] and Blazquez et al.'s .[*sic*] methods to design a product and use Berlioz et al.'s method to express the protein in an eukaryotic cell.

This rejection respectfully is traversed.

The rejected claims

Claim 24 includes the method of claim 1 and recites that the nucleic acid molecules are in viral vectors and the titer of each set of vectors is assessed at step (b).

Claim 28 recites that the at step (f) in the method of claim 27 the titer of the viral vectors in each set of cells is determined; and claim 29 recites that in the method of claim 28, the target protein is a protein involved in viral replication..

Analysis

As discussed above, the combination of teachings of Ladner *et al.*,, Wells *et al.*, and Pederson *et al.*, fails to teach or suggest teach any or all limitations of independent claims 1 and 27 or any of the pending claims, and hence fails to teach or suggest any or all elements of the rejected dependent claims. Berlioz *et al.* fails to cure the deficiencies in the teachings of these references since Berlioz *et al.* does not teach or suggest a method that includes producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, where all nucleic acid molecules in a set encode the same modified protein and the proteins are modified along their full-length or the full-length of a domain (step (a)); arraying cells containing the nucleic acid molecules such that the identity of the encoded protein is known *a priori*(step (b)), and/or individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)), nor any other steps or elements of the methods as claimed.. Therefore the combination of teachings of Ladner *et al.*, Wells *et al.*, Pederson *et al.*, and Berlioz *et al.* does not result in the methods of claims 7, 24 and 27-29.

The rejection of claims 25, 26, 32 and 33

Claims 25, 26, 32, and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. in view of Wells et al. in view of Pedersen et al. in view of Berlioz et al. as applied to claims 1-3, 5-10, 14, 15, 17-23, 27, 32, 33, 43 and 44 above, and further in view of

Drittanti et al. (Gene Therapy (2000) Volume 7, pages 924-929) because Drittanti et al. teaches real time virus titering (page 925); using tagged replication and expression enhancement (page 926, right column); and where the process is automated and computer controlled (page 925, left column under Figure 1), which are elements of claims 25, 26, 32 and 33. The Examiner concludes that it would:.

> have been obvious for one of ordinary skill in the art at the time of invention to modify the methods of Ladner et al., Wells et al., Pedersen et al., and Berlioz et al. with Drittanti et al. to gain the benefit of determining the effectiveness of viral vectors. Berlioz et al. teach that one of his goals is to create an effective and stable viral vector (column 1, lines 10-17). Part of their method requires that they assess the titer of the viral vectors after transmission. Drittanti et al.'s method provides further insight into the stability and efficacy of the vector by offering real time titering. Thus one of ordinary skill in the art would be motivated to combine the methods of Ladner et al., Wells et al., Pedersen et al., and Berlioz et al. with Drittanti et al. in order to gain the benefit of assessing the stability and efficacy of viral vectors.

This rejection respectfully is traversed.

Without conceding whether Drittanti *et al.*, which is authored by inventors of the instant application, is effective as prior art against any claims in this application, it does teach real time tittering. Drittanti *et al.*, however, does not teach any elements in the independent claims missing from the combination of teachings of Ladner et al. in view of Wells et al. in view of Pedersen et al. in view of Berlioz et al. Therefore, the combination of teachings of Drittanti *et al.* with those of Ladner et al. in view of Wells et al. in view of Pedersen et al. in view of Berlioz et al. does not result in the methods of any of claims 25, 26, 32 and 33.

The rejection of claims 30 and 31

Claims 30 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner et al., Wells et al. and Pedersen et al., as applied to claims 1-3, 5-10, 14, 15, 17-23, 27, 43 and 44 above, and further in view of Persson et al. (Journal of Virology (1985) Volume 54, pages 92-97) because Persson *et al.* is alleged to teach a method that uses a Hill analysis for determining the rate in which host cells are infected with viruses (abstract, page 94, left column). The Examiner concludes that:

> [i]t would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ladner et al., Wells et al., and Pedersen et al. with the method of Persson et al. to gain the benefit of determining if the plasmids or vectors are infecting the host cells. Ladner et al. and Wells et al. teach creating host cells with desired nucleic acids. In such a method, it would be desirable to determine the rate of infection in order to determine how to structure an experiment (e.g., incubation times, concentration, etc.). Persson et al. provide a method of determining the rate of infection. Thus one of ordinary skill in the art would be motivated to combine the methods of Ladner et al., Wells et al., and Pedersen et al.

with the method of Persson et al. to gain the benefit of determining the rate of infection of host cells to structure his experiments.

This rejection respectfully is traversed.

Claim 31, which is dependent on claim 30, which includes the elements a)-g) as discussed above, and further includes recites that the "performance of the screened proteins is evaluated by a Hill analysis. Persson *et al.* does not teach or suggest a Hill analysis as claimed, nor does Persson *et al.* teach or suggest the elements of claim 30, missing from the combination of teachings of Ladner *et al.*, Wells *et al.*, and Pederson *et al.* Therefore, the combination of teachings of Ladner *et al.*, Wells *et al.*, Pederson *et al.* and Persson *et al.* does not result in the methods of claims 30 and 31. Therefore for these reasons and those discussed above, the Examiner has failed to set forth a *prima facie* case of obviousness of any of the pending claims.

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In view of the above, reconsideration and allowance respectfully are requested.

Respectfully submitted,

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